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25. Steroid Action in CNS and Anterior Pituitary—II

FUNCTIONAL AND ORGANIZATIONAL ASPECTS OF GONADAL STEROIDS IN THE PIG BRAIN

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SUMMARY

Sexual differentiation of the brain-pituitary system of pigs occurs prenatally, since it is possible to alter the positive feedback response of LH to oestrogen in the postnatal female by prenatal treatment with testosterone (T). In the adult male pig feedback responses of LH to either T or 5α-dihydrotestosterone (5α-DHT) are mediated via the amygdala (AMY) and hypothalamus. Microimplants or microinjections of either substance into the AMY or mediobasal hypothalamus show that both, T and 5α-DHT can stimulate and inhibit LH-secretion. Catechol-oestrogens are one link between androgens metabolizable to oestrogens and LH control mechanisms. An altogether different action of steroids in the pigs brain is given by the boar pheromone 5α-androst-16-en-3-one and T when applied as an aerosol to the pigs nasal mucosa. Both androgens change olfactory bulb unit activity. Transmission through the lateral olfactory tract may eventually reach the limbic hypothalamic system.

INTRODUCTION

It is undisputed, that steroids exert part of their functions at the brain level. Important assignments in the organizational phase of the brain and endocrine development have been given to steroids. Steroid–brain-interactions continue during adulthood, where they are considered essential mediators of brain-pituitary-function. Despite a wealth of evidence on the role of steroids, major gaps of knowledge await answers to questions such as: When do steroids exert their organizational power in species that are differentiated already at birth into endocrine males and females? Can we recognize a definite role of steroids with respect to pituitary release of hormones, e.g. inhibitory or stimulatory? Where are functionally receptive sites for steroids located within the brain? Are pathways and mediators of steroid function known? Can steroidal pheromones affect brain function? We shall present the results of experiments by means of which our group tackled some of these questions in the miniature pig.

ORGANIZATIONAL ASPECTS OF STEROIDS DURING ENDOCRINE DIFFERENTIATION

The initial findings in the rat [1], that the brain is involved in sexual differentiation was later confirmed by morphological [2, 3] and neurophysiological [4] evidence. In other species the endocrine sex is not as easily accessible to manipulation as in the rat. However, the female miniature pig will within the first two weeks of life respond with a surge of luteinizing hormone (LH) to a single intramuscular injection of oestradiol-benzoate. The male does not (Fig. 1) respond, although following injection circulating oestradiol levels were similar in females and males [5]. We can assume therefore, that the processes leading to a sexually dimorphic response to oestradiol occur prior to parturition in the miniature pig. To determine when this occurs we injected testosterone directly into the fetus on days 40 or 50 of fetal life (pregnancy: 115 days). Clitoral enlargement occurred in all female offspring and was a sign that testosterone had a masculinizing effect. When these miniature piglets were treated within 2 weeks of birth with oestradiol-benzoate for a positive feedback on LH, 22 of 23 piglets (11 females, 12 males) failed to respond with a surge of LH. All sham treated and control females displayed significantly elevated plasma LH levels after oestradiol-benzoate application (Fig. 1 and Fig. 2). None of the males responded. Hence it can be suggested that the stage for sexual differentiation of the positive feedback is set during the first half of fetal life but can still be altered, even when differentiation of the external genitalia is largely completed.

FUNCTIONAL ASPECTS OF STEROID FEEDBACK ON THE BRAIN IN THE ADULT MALE

Slow progress has been made in recent years to reduce the size of the black box, which is still characteristic of our knowledge on steroid feedback in the male [6, 7]. If we are to attempt to answer some of the questions raised in the introduction, we first must assign a defined role for steroids in a system such as the pituitary LH-secretion and then investigate further questions. We therefore applied two different dose levels of gonadal steroids intramuscularly (i.m.) to castrated male miniature pigs and observed changes in plasma LH 24 h later (Fig. 3). While testosterone as well as oestradiol-17β exerted a negative feedback on LH secretion at both dose levels, we were

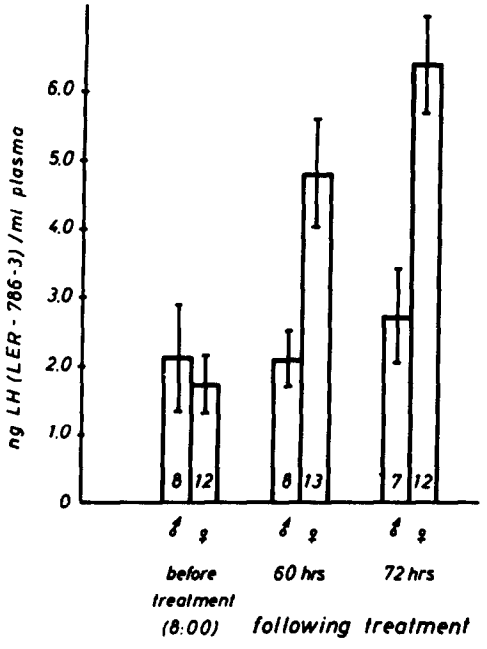

Fig. 1. Positive feedback on LH in the plasma of female, but not male miniature pigs after treatment with oestradiol-benzoate (0.6 mg/kg BW) at 2 weeks of age (data from Elsaesser, Parvizi and Ellendorff, 1978 [5]).

surprised to find a dual response of 5α-dihydrotestosterone (5α-DHT), with a negative feedback at the higher dose and a positive feedback at the lower dose. A positive feedback of 5α-DHT on LH has so far not been reported from other species. Although circulating 5α-DHT- and testosterone-levels were considerably higher in 5α-DHT- and testosterone-treated animals than in the intact adult male, the phenomenon was of sufficient interest to us to attempt to localize the positive responses in the brain.

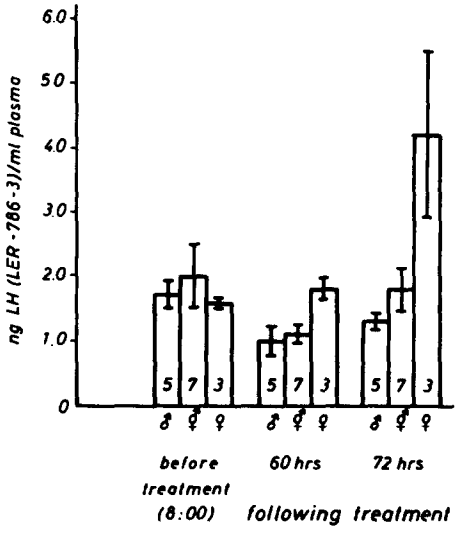

Fig. 2. Absence of positive feedback on LH in the plasma of male (♂) and masculinized female (♀) piglets after treatment with 0.6 mg oestradiol benzoate/kg BW at 2 weeks of age. Piglets had been treated on day 40 of fetal life with testosterone (0.2 ml Testoviron Depot 100. Schering). Sham-injected females (♀) displayed a surge in LH. (Elsaesser, Parvizi and Ellendorff, unpublished).

Implantation of crystalline 5α-DHT, testosterone or cholesterol into the basolateral amygdala and mediobasal hypothalamus (MBH) of a series of castrated males via exchangeable stainless steel tubing resulted again in a positive feedback of 5α-DHT at both sites between day 1 and 12 after implantation when the implants were still *in situ*. Testosterone lowered plasma LH when implanted into the amygdala and stimulated when implanted into the MBH [8]. Later a more refined technique of microinjection also provided us with strong evidence, that one site of the positive feedback of 5α-DHT is the amygdala (Fig. 4).

The sampling schedule allowed us to detect the positive feedback at 24 h and more pronounced at 48 h after local microinjections into the amygdala. Testosterone or oestradiol-17β did not evoke significant changes in LH (Fig. 4).

A further experiment (Parvizi, Ellendorff, unpublished), was performed to substantiate the role of steroids in the amygdala, namely to establish, whether or not steroids may alter the response of the amygdala to electrical stimulation and thus relay different information to the pituitary. Fully awake castrated male miniature pigs were microinjected into the basolateral and cortical-medial amygdala with either testosterone, 5α-DHT, oestradiol-17β or solvent or received no injection 3.5 h prior to electrical stimulation via a bipolar concentric electrode, that was attached to the microinjection cannula. At weekly intervals each animal received a different treatment until it had obtained all in random order. The results of the experiments supported our hypothesis, since prior treatment of the amygdala with steroids definitely changed the pattern of response (Fig. 5): where stimulation alone caused LH to decrease, prior treatment with 5α-DHT or oestradiol-17β abolished the decline, again indicating the facilitatory role of 5α-DHT in the amygdala. When testosterone was given prior to electrical stimulation, an elevation of LH was observed.

The conclusion we could draw from these series of experiments was, that 5α-DHT can evoke both negative and positive feedback responses of LH. Locations of positive 5α-DHT-function must be within the amygdala and the hypothalamus. With respect to testosterone, the mediobasal hypothalamus represents one site where positive responses originate, while negative effects are located in the amygdala. All steroids are able to eliminate the suppressive effect of electrical stimulation on LH secretion, which suggests, that the responsiveness of amygdala neurons to electrical stimulation is altered by steroids. These experiments also show a large variability in the responses obtained under different experimental protocols, which are influenced by dose, time and site of steroid application.

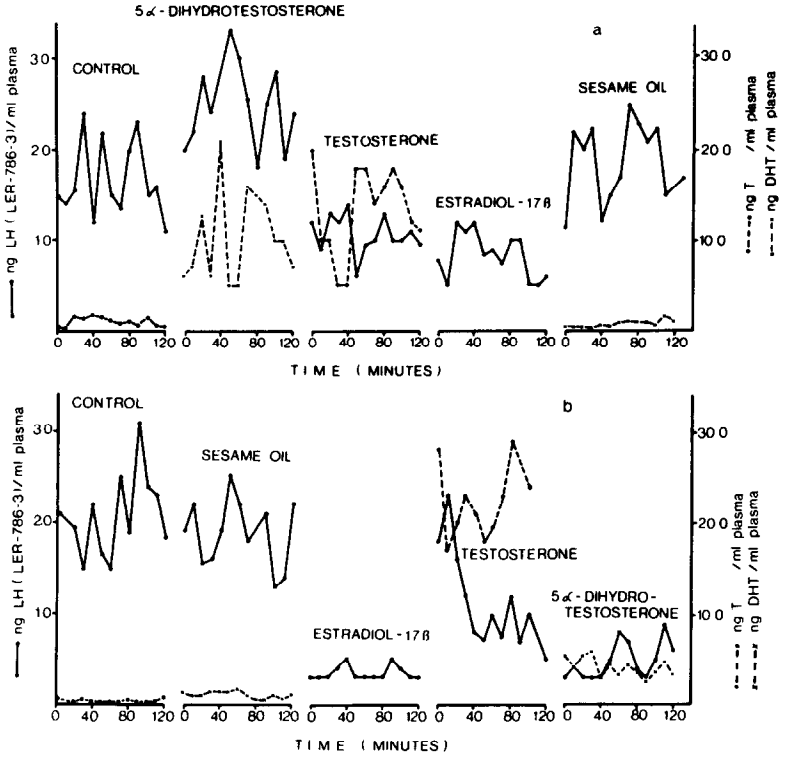

Fig. 3. Responses of plasma LH in individual animals 24 h after i.m. steroid treatment. Included are circulating testosterone (T) or 5α-dihydrotestosterone (DHT)-levels: (a) T and DHT = 6 mg/kg BW; 17β-oestradiol (E_2) = 0.6 mg/kg BW; sesame oil = 2 ml; (b) T and DHT = 15 mg/kg BW; E_2 = 1.5 mg/kg BW. Note the paradoxical plasma DHT-levels in a. and b. after DHT-treatment. (From Parvizi, Elsaesser, Smidt and Ellendorff, 1977 [8]).

2-HYDROXY-OESTRADIOL, A PUTATIVE MEDIATOR OF STEROID FUNCTIONS IN THE BRAIN

Testosterone can be aromatized to oestradiol [9]. 2-hydroxy-oestradiol (2-OHE_2) is a metabolite of oestradiol and has been found to occur in the brain [10]. Position 2-hydroxylated oestradiol competes with higher affinity for catechol-o-methyltransferase than catecholamines [11, 12]. The subsequent inhibition of catecholamine metabolism and the expected accumulation of norepinephrine could represent one pathway of steroid-neurotransmitter interaction. To test this assumption we microinjected 2-OHE_2 into the amygdala [13]. The resulting inhibition of LH

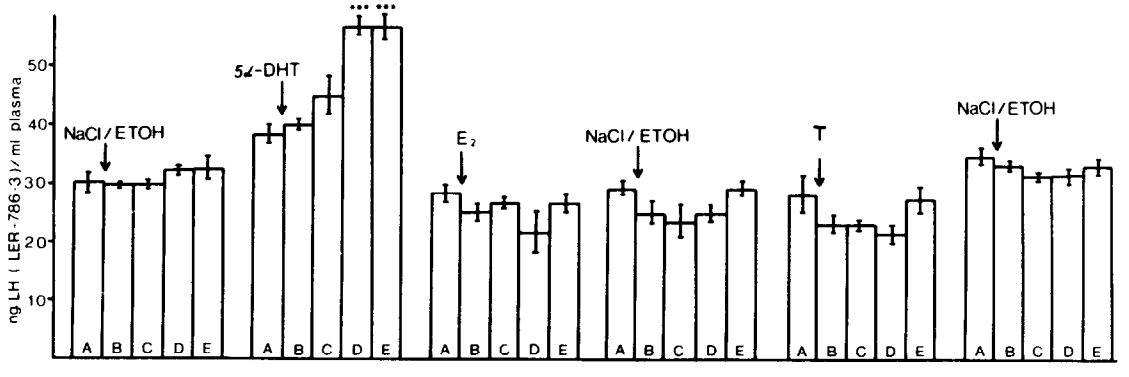

Fig. 4. Responses of an individual animal to microinjection into the amygdala of 2 μl NaCl-EtOH (7 ml 0.9% NaCl + 3 ml 20% ethanol), 60 ng 5α-dihydrostestosterone (DHT), 6 ng 17β-oestradiol (E_2) and 60 ng testosterone (T) each dissolved in 2 μl NaCl-EtOH. Arrows indicate time of injection. Number of samples per block: seven to nine obtained at 15 min intervals. Each column (B–E) was compared with the corresponding control period (A) by Student's *t*-test: ***, $P < 0.001$. A = 0–2 h before microinjection; B = 0–2 h after microinjection; C = 2–4 h after microinjection; D = 24–25.5 h after microinjection; E = 48–49.5 h after microinjection; brackets, ±S.E.M. (From Parvizi, Elsaesser, Smidt and Ellendorff, 1977 [8]).

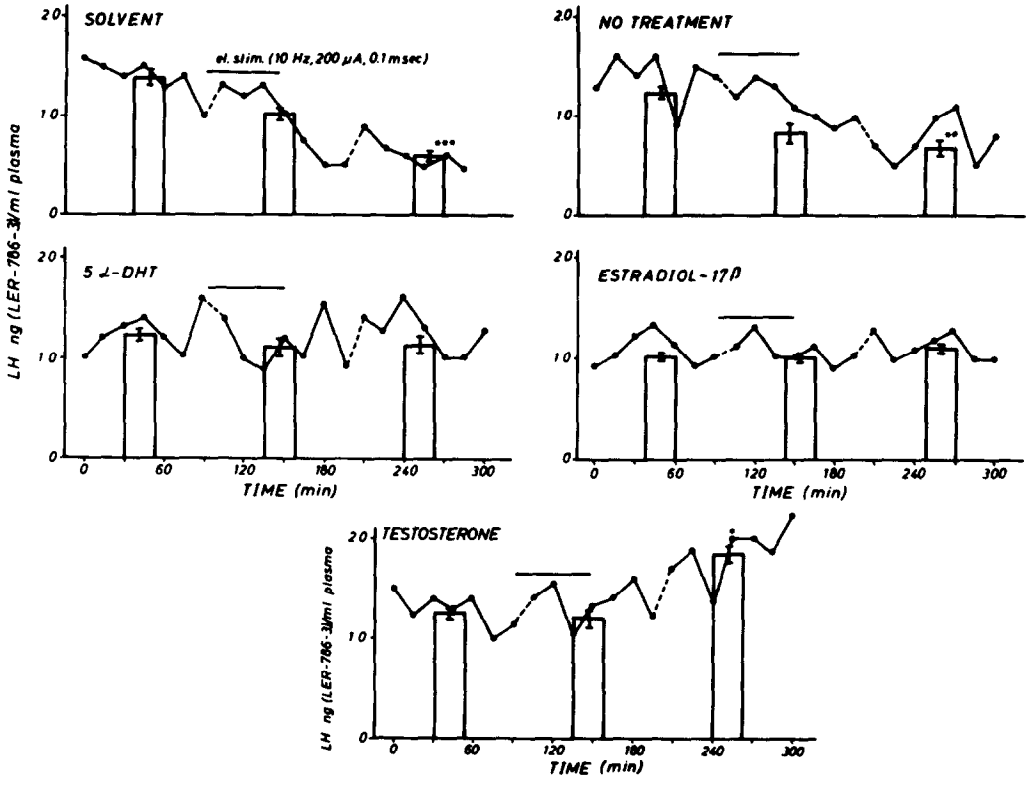

Fig. 5. Abolishment of electrical stimulation induced inhibition of LH secretion by prior microinfusion of steroids. Site of electrical stimulation and infusion: Amygdala. Microinfusion took place 3.5 h prior to the onset of electrical stimulation. Solvent: 2 μl NaCl-EtOH (7 ml 0.9% NaCl) + 3 ml 20% ethanol); 5α-DHT (5α-dihydrotestosterone) and testosterone: 4 ng/kg BW; 17β-oestradiol: 0.4 ng/kg BW. Data from one fully awake animal treated at weekly intervals. Bars represent the mean ± S.E.M. of the pooled data over 60 min periods $*P < 0.05$, $***P < 0.001$. Analysis of variance, followed by Student's *t*-test. (Parvizi and Ellendorff, unpublished).

by 2 h and by 4 h after microinjection provided clear evidence that 2-hydroxylation can be one mechanism by which testosterone and its metabolites affect LH (Fig. 6). From unpublished data we know finally, that norepinephrine microinjected at 10^{-6} ng into the amygdala can inhibit LH secretion, which is a further link on the still long way to explain mediators and pathways of steroid action in the brain.

PHEROMONES AFFECT BRAIN FUNCTION

The final aspect of steroid function in the brain which we wish to discuss is so far limited uniquely to the pig, where a testicular steroid, 5α-androst-16-en-3-one, has been isolated and identified as a pheromone [14, 15]. Major sites of steroid accumulation are the submaxillary glands from where it is transmitted to the environment during excitement and sexual

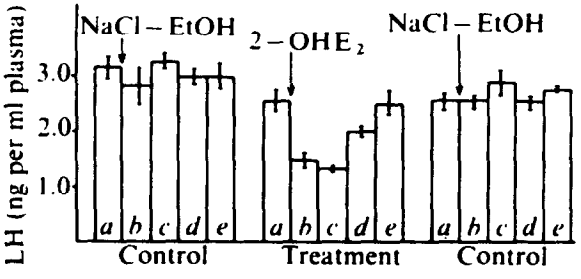

Fig. 6. Plasma LH (mean ± S.E.M.; LER-786-3) after microinjection of 2-OHE_2 into the amygdala of an individual orchidectomized adult miniature pig. NaCl-EtOH: 2 μl of a stock made from 7 ml 0.9% NaCl and 3 ml 20% EtOH; 2-OHE_2; 2 μl NaCl-EtOH containing 60 ng 2-OHE_2; number of samples per block: seven to nine, obtained at 15 min intervals. Each column (b–e) was compared with the corresponding control period (a) by Student's *t*-test, 2-OHE_2 treatment resulted in significant decrease in columns (b) and (c), ($P \leq 0.001$) and in column (d), ($P \leq 0.01$). a, 0–2 h before microinjection; b, 0–2 h after microinjection; c, 2–4 h after microinjection; d, 24–25.5 h after microinjection; e, 48–49 h after microinjection. (From Parvizi and Ellendorff, 1975 [13]).

Fig. 7. Pig pheromone (5α-androst-16-en-3-one, Δ-16) and testosterone (T) affect extracellular unit activity in the mitral cell layer of the olfactory bulb in the pig. Substances were passed by the olfactory mucosa as aerosols. A = Amylacetate, P = Pyridine, B = Benzol (MacLeod, Reinhardt and Ellendorff, unpublished).

arousal and is able to induce estrous behavior in estrous sows. Although 5α-androst-16-en-3-one has been shown to bind onto receptors of the olfactory mucosa [16], it has never been shown to actually alter brain or even olfactory bulb function as the likely first site of action within the brain. To show, whether or not 5α-androst-16-en-3-one is able to alter olfactory bulb activity, we used the anesthetized pig. A number of substances including 5α-androst-16-en-3-one and testosterone were passed over the nasal mucosa. Extracellular recordings from the mitral cell layer, which had been identified by the field potential technique was performed. Results (Fig. 7) clearly demonstrate, that 5α-androst-16-en-3-one, but also testosterone containing aerosols can alter unit activity in the mitral cell layer, which projects directly into the lateral olfactory tract. The lateral olfactory tract of the pig directly projects into the amygdala and orthodromically into the mediobasal hypothalamus (Konda, Ellendorff, unpublished). Three types of responses could be distinguished: non-responsive neurons, indiscriminatory neurons and discriminatory neurons, which recognize one steroid, but not the other. Responses occurred in all animals, males, castrated males or diestrous females.

Thus, it could be demonstrated, that steroids may alter brain unit activity via the olfactory bulb.

CONCLUSIONS

With reference to the questions raised in the introduction, we have established organizational as well as functional tasks for steroids on the brain. During the first half of fetal development testosterone treatment leads to endocrine masculinization of genetically female piglets. In the adult male pig 5α-DHT may assume a dual role of positive and negative feedback on LH-secretion. Sites of the positive feedback can be located in the amygdala and mediobasal hypothalamus. 2-hydroxy-oestradiol can be considered as a putative link between steroids and catecholamines leading to altered LH secretion. A steroidal pheromone, 5α-androst-16-en-3-one, but also testosterone affect brain function via electrical changes in the olfactory bulb.

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